IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No.	:	10/699,511	Confirmation No. 3571
Applicant	:	Bennett, George N.	
Filed	:	October 31, 2003	
TC/A.U.	:	1637	
Examiner	:	Calamita, H.	
Docket No.	:	31175413-002002 (22055)	
Customer No.	:	51738	
Entitled	:	Method for Assembling PCR Fragments of DNA	

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

APPEAL BRIEF PURSUANT TO 37 C.F.R. §1.191

Appellants filed the amended Appeal Brief on January 11, 2007. In response to the Notification of Non-Compliant Appeal Brief mailed March 11, 2008, Appellants file this amended Appeal Brief.

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1. **REAL PARTY IN INTEREST**

The real party in interest is Rice University, Office of the General Counsel – MS 94, P.O. Box 1892, Houston, TX 77251-1892.

2. **RELATED APPEALS AND INTERFERENCES**

Appellants know of no other appeal or interference that will directly affect, or be directly affected by, or that will have a bearing on the Board's decision in the pending appeal.

3. **STATUS OF CLAIMS**

Claims 1-7 are pending in this application and are the claims from which this Appeal is taken. These claims were finally rejected in an Office Action dated July 26, 2007. A copy of the pending claims is attached as APPENDIX A.

4. **STATUS OF AMENDMENTS**

Applicant submitted an Amendment After Final Rejection on September 26, 2007 subsequent to the Final Rejection mailed July 26, 2007. Also on September 26, 2007 Applicants filed a Notice of Appeal along with a Pre- Appeal Brief request for Review. In an Advisory Action Before The Filing of an Appeal Brief mailed October 16, 2007, the Patent Office indicated that the Amendment of September 26, 2007 did not place the Application in condition for allowance, but that the Amendment of September 26, 2007 would be entered for the purposes of appeal.

5. **SUMMARY OF CLAIMED SUBJECT MATTER**

The invention generally relates to a method of quickly assembling PCR fragments on a solid support using a unique 3' overhang system and DNA repair enzymes. When the assembly is penultimately complete, a recombinase is used to simultaneously remove the DNA from the solid support and circularize it in a single step. **This is the first showing in the art that recombinases can be successfully employed on substrates tethered to a solid support.** *See* Claim 1; Specification page 2, lines 12 through 20 and page 2, line 27 through page 3, line 24 and page 5, lines 1 through 27; Example 2.

More particularly, the invention relates to a method of assembling PCR fragments by: a) synthesizing a PCR fragment with a first and second primer, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and the first PCR fragment has a first site specific recombinase site; b) treating the PCR fragment with a DNA repair enzyme to generate the 3' overhang and immobilizing the PCR fragment on a solid support; c) making a second PCR fragment with third and fourth primer, that each comprise a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang; d) treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang; e) annealing and ligating the first and second PCR fragments; f) optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, the last PCR fragment comprising a second site specific recombinase site; and g) simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step. *See* Claim 1; Specification page 2, lines 12 through 20 and page 2, line 27 through page 3, line 24 and page 5, lines 1 through 27; Example 2.

Additional information is provided in the following Table.

Table – Summary of Claimed Subject Matter

Claim Language	Specification Support
Claim 1: A method of assembling PCR fragments	See title (METHOD FOR ASSEMBLING PCR FRAGMENTS OF DNA)
Claim 1: making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and wherein the first PCR fragment comprises a first site specific recombinase site	See original claim 1 ("making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and wherein the first PCR fragment comprises a first site specific recombinase site"). See also ¶ 8, 22, 23 24 (each discussing modified nucleotide), and ¶ 37 (discussing making oligonucleotides [primers]).
Claim 1: treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang and immobilizing the first PCR fragment on a solid support or vice versa	See original claim 1: ("treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang and immobilizing the first PCR fragment on a solid support or vice versa") See also ¶ 4, 22, 24, 25 (last sentence), 37, 38 19 (each discussing the overhang) and 4, 8, 9, 22 (discussing repair enzyme)
Claim 1: making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang	See original claim 1 ("making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang"). See also ¶ 8, 22, 23 24 (each discussing modified nucleotide), and ¶ 37. See also ¶ 38 (discussing second pair of primers).
Claim 1: treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang	See original claim 1 ("treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang") See also ¶ 4, 22, 24, 25 (last sentence), 37, 38 19 (each discussing the overhang) and 4, 8, 9, 22 (discussing repair enzyme)
Claim 1: annealing and ligating the first and second PCR fragments	See original claim 1 ("annealing and ligating the first and second PCR fragments") See ¶ 4, 8, 20, 24, 25, 41 (discussing ligation or joining reaction)
Claim 1: optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises a second site specific recombinase site	See original claim 1 ("optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises a second site specific recombinase site") See also ¶ 4, 10, 25 48 (discussing sequential addition of fragments)

Claim Language	Specification Support
Claim 1: simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step	See original claim 1 ("removing and circularizing the assembled fragment from the solid support with a site specific recombinase") See also ¶ 11, 17, 18, (discussing removal and circularization), 26 ("The released fragment is in a circular form which allows it to be efficient for transformation as it is removed from the scaffold") and 41 ("Cre recombinase is added to the beads which have C bound, in order to circularize C using the loxP sites; circularizing detaches C from the beads and forms a product that can be used directly in transformations").
Claim 2: method of claim 1, where one of the PCR fragments comprises an origin of replication and a selectable marker	See ¶ 27 (discussing additional signals can be incorporated into the assembled DNA fragments, such as an origin of replication, and a selective marker)
Claim 3: method of claim 1, wherein the first PCR fragment or the last PCR fragment comprises an origin of replication and a selectable marker	See ¶ 27 (discussing signals can be incorporated into the assembled DNA fragments, such as an origin of replication, and a selective marker, and signals will be contained in the first or last PCR fragment)
Claim 4: method of claim 1, wherein the site specific recombinase is CRE and the site specific recombinase site is lox	See ¶ 11, 17, 26, 41 and Fig. 4 (discussing one site specific recombinase system is the cre-lox system)
Claim 5: method of claim 1, wherein the nucleotide is deoxyuridine and the DNA repair enzyme is uracil-DNA-glycosylase followed by T_4 endonuclease V	See ¶ 8, 23, 37, 38, 39, and 45 (discussing the nucleotide is deoxyuridine and the commercially available repair enzymes used are uracil-DNA-glycosylase (4) and T_4 endonuclease V)
Claim 6: method of claim 5, wherein the assembled DNA is greater than 30 kb	See ¶ 20 (discussing the most efficient way to obtain larger fragments, >30 kb, is by PCR amplification using specific primers to precisely define the ends and ligating the component fragments in a defined order) and Claim 6
Claim 7: method of claim 5, wherein the assembled DNA is greater than 30, 40, 50, 75, 100, 125, 150, 200, 300, 350, 400, 450, 500, 750, 1000, or 1500 kb	See ¶ 20 (discussing the most efficient way to obtain larger fragments, >30 kb, is by PCR amplification using specific primers to precisely define the ends and ligating the component fragments in a defined order) and Claim 7

6. **GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL**

The Examiner has twice rejected claims 1-7 as obvious over Watson (Biotechniques, 1997) and Elledge (US5851808), in view of Stahl (Biotechniques, 1993).

7. **ARGUMENT**

The Examiner has rejected claims 1-7 as obvious over Watson, Elledge and Stahl.

i) CITED ART

Watson teaches a DNA fragment assembly method using 3' overhang and repair enzyme. Watson does not teach modification by using recombinase, nor a recombinase site, nor application to a solid support. Instead, Watson performs all assembly in solution using traditional enzymes such as ligase.

Elledge is a patent teaching a method of combining two vectors using a recombinase, wherein each vector has a recombinase site and **one of the vectors must have a conditional origin of replication**. Conspicuously lacking from Elledge is any mention of solid support. Instead, all reactions are done in solution. Elledge apparently did not consider including this allegedly obvious modification of his method in his patent application.

Stahl teaches assembly of linear DNA from short oligonucleotides on a solid support. There is no mention in Stahl of recombinase, nor does Stahl complete the process on the solid support. Instead, the partially assembled DNA is eluted from the support, then the remaining assembly is performed in solution using traditional enzymes such as ligase.

ii) WHAT IS MISSING IN THE CITED ART

Nowhere in the cited art is a **recombinase used on a solid support**. The only enzymes used on solid supports in the cited art are simple enzymes such as restriction endonucleases and ligases—**neither of which are topologically sensitive** enzymes. The art does not teach the use of a recombinase on a solid support—nor does the art suggest a reasonable expectation of success for so doing.

iii) PRIMA FACIE OBVIOUSNESS CASE HAS NOT BEEN SHOWN

The *prima facie* obviousness burden lies on the Examiner to show at least the following:

1) that the art teaches every element of the claimed invention, 2) that there is a motivation to combine or modify the art,[1] and 3) that there is a reasonable expectation of success in making that combination or modification. While the expectation of success need not be absolute, there does need to be a **reasonable** expectation of success. *Takeda Chem. Indus. v. Mylan Labs., Inc.*, 417 F. Supp. 2d 341, 371 (Fed. Cir.2006) ("While a reasonable expectation of success must be shown, in order to show *prima facie* obviousness it is not necessary to show that success was absolutely predictable."); *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991) (discussing the obviousness case and stating that one element is "whether the prior art would also have revealed that . . . those of ordinary skill would have a reasonable expectation of success.").[2]

iv) MISSING ELEMENT NOT FOUND IN THE CITED ART

None of the cited art shows recited step g: "g) simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step".

Applicants have reviewed the cited art over and over and can find no instance where DNA is simultaneously removed and circularized from a solid support with a recombinase. Indeed, the **only** cited art mentioning recombinases is Elledge, and Elledge fails to mention solid supports at all.

There is simply no mention in the cited art of using a recombinase on a solid support. Examiner's argument to the contrary devolves to saying that 1) recombinases are in the art, and 2) solid supports are in the art, therefore 3) using recombinases on solid supports is in the art. However, the element is "using recombinases on a solid support," **not either alone**.

Even in the wake of *KSR Int'l Co. v. Teleflex Inc.* (127 S. Ct. 1727 (U.S. 2007)), Applicants know of no legal principle that suggests that a *prima facie* case can be maintained

[1] KSR did not negate the motivation to combine test, but only cautioned against its rigid application. *KSR Int'l Co. v. Teleflex Inc.*,127 S. Ct. 1727, 1741 (U.S. 2007) ("When it first established the requirement of demonstrating a teaching, suggestion, or motivation to combine known elements in order to show that the combination is obvious, the Court of Customs and Patent Appeals captured a helpful insight. . . . a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.. . . it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.").

[2] *See* also *MPEP* 2143.02 **entitled** "Reasonable Expectation of Success Is Required".

where a claimed element is not found in the prior art. Thus, in the absence of this element, the obviousness rejection cannot be maintained because the *prima facie* case is not made.

v) EXAMINER FOCUSES ON UNCLAIMED ELEMENT

The Examiner states that "it is well established that Cre/lox will simultaneously recombine and circularize plasmid DNA."[3] This is **not** what the claim recites. The claims recites:

g) "simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step".

To simply the element and focus the reader the element is paraphrased:

"removing and circularizing DNA on a solid support with a recombinase"

The Examiner has not found this element because recombinase is **never** used on a solid support in the cited art, therefore, it is **impossible** to find that recombinase simultaneously removes and circularizes DNA on a solid support in the art.

vi) EXAMINER FAILS TO SHOW A REASONABLE EXPECTATION OF SUCCESS

The Examiner states "It is well established that Cre/lox will simultaneously recombine and circularize plasmid DNA. It is therefore not unreasonable to expect success when using Cre/lox to simultaneously recombine and circularize DNA which is attached to a substrate."[4]

However, a closer look at this statement shows that it is merely conclusory and has **not** articulated any rationale to support an assertion of reasonable expectation of success. The statement in fact devolves to a statement of recombinase activity and a conclusion that based on the activity that one would expect it to function on a solid support.

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[3] Advisory Action, 10/26/07. *See* also Office Action, 7/26/07, p. 2 (stating the same as the Advisory Action), and Office Actions, dated 2/22/07, 12/27/06 10/03/06, 7/27/06, and 2/22/06, (none of which even *mention* the reasonable expectation of success although it is part of the *prima facie* case, and which are mere "cut and pastes" of the original argument, repeating the same spelling errors and legal deficiencies).

[4] Advisory Action, 10/26/07.

The Examiner is only saying that **"Cre/lox is a recombinase and can be expected to function on a solid support."**

This is **not** "articulated reasoning with some rational underpinning" as required by KSR. *KSR Int'l Co,*, 127 S. Ct. at 1741 (U.S. 2007).

Therefore, Examiner merely assumes that the recombinase method of Elledge **can** be applied to the solid support method of Watson with a reasonable expectation of success. Examiner has not pointed to any actual statements in the art supporting this assumption, nor has Examiner provided any declaratory evidence for same, nor has Examiner even provided a logical rationale for assuming same.

In an unpredictable art, there is simply no basis for **assuming** that the assemble method could be successfully applied to recombinase enzymes known to be sensitive to topology. To do so, is sheer speculation, which we refer to as an inappropriate hindsight determination of obviousness.

Further, conclusory assertions without an articulated rationale or factual basis of support is **insufficient as a matter of law**. *KSR Int'l Co,*, 127 S. Ct. at 1741 (U.S. 2007) ("To facilitate review, this [obviousness] analysis should be made explicit. '[R]ejections on obviousness grounds cannot be sustained by **mere conclusory statements**; instead, there must be some **articulated reasoning with some rational underpinning** to support the legal conclusion of obvious-ness'") (citation omitted) (emphasis added).

Without a rationale and/or evidence to support a reasonable expectation of success, the *prima facie* case is not made.

vii) MERE ARGUMENTS ARE NOT EVIDENCE

Personal knowledge or mere argument is **not** competent evidence. *Fiers v. Revel*, 984 F.2d 1164 (Fed. Cir. 1993) (holding that "the Board did not err in determining that Fiers presented no convincing evidence" where applicant only showed "argument ... 'unsupported by competent evidence, entitled to little or no weight and ... unpersuasive in any event.'"); *In re Juillard*, 476 F.2d 1380 (C.C.P.A.) ("arguments cannot take the place of evidence"). The Examiner has provided no evidence at all to indicate that a recombinase can be expected to work on a solid support, merely unsupported conclusory speculation.

viii) TWO ELEMENTS OF *PRIMA FACIE* CASE MISSING

Therefore, the Examiner has not made a *prima facie* case of obviousness since 1) a claimed element is missing (using a recombinase on a solid support) and 2) a reasonable expectation of success is not shown.

* * *

In contrast, Applicants prove that there is **no** reasonable expectation of success, as follows:

ix) RECOMBINASES ARE TOPOLOGICALLY SENSITIVE

Recombinases are topologically sensitive. The literature is replete with evidence to this effect, as shown in the last Response to Office Action, mailed September 26, 2007 and repeated herein in part for convenience:

> Kilbride EA, et al., Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system, J Mol Biol. 355(2):185-95 (2006) (**Many natural DNA site-specific recombination systems achieve directionality and/or selectivity by making recombinants with a specific DNA topology. This property requires that the DNA architecture of the synapse and the mechanism of strand exchange are both under strict control.** Previously we reported that Tn3 resolvase-mediated synapsis of the accessory binding sites from the Tn3 recombination site res can impose topological selectivity on Cre/loxP recombination. Here, we show that the topology of these reactions is profoundly affected by subtle changes in the hybrid recombination site les. Reversing the orientation of loxP relative to the res accessory sequence, or adding 4 bp to the DNA between loxP and the accessory sequence, can switch between two-noded and four-noded catenane products. By analyzing Holliday junction intermediates, we show that the innate bias in the order of strand exchanges at loxP is maintained despite the changes in topology. We conclude that a specific synaptic structure formed by resolvase and the res accessory sequences permits Cre to align the adjoining loxP sites in several distinct ways, and that resolvase-mediated intertwining of the accessory sequences may be less than has been assumed previously.").[5]

The art establishes that recombinases are topologically sensitive. This **fact** has not been rebutted.

x) TOPOLOGICALLY SENSITIVE RECOMBINASES ARE PREDICTED TO NOT FUNCTION ON TETHERED SUBSTRATES

Because recombinases are topologically sensitive they are always used in solution (or cells) where the DNA molecules can freely move around to assume the complex knotted forms

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[5] The remainder of the citations can be found in the record (Response to Office Action, mailed 9/26/2007) and in Appendix B, and there is no need to repeat this well-known fact herein.

required during the recombinase reaction. It is for this reason that Watson did not think to apply recombinases to his method (*see* Bennett Declaration by co-author of Watson paper) and is probably why Elledge is completely silent regarding using his method on a solid support.

This **rationale** has not been rebutted.

xi) Declaratory Evidence Shows That Topologically Sensitive Recombinases are not Expected to Function on Substrates Tethered to a Solid Support

Dr. George N. Bennett is a person of at least ordinary, and probably better than ordinary, skill in the art. His Declaration states that one ordinary skill in the art would not have thought recombination on a solid support would succeed due to the topological restraints imposed by attaching one end to a solid support:

> Prior to the present invention, the ability of the CRE protein to function on immobilized DNA was unknown. The use of immobilized DNA for the *Cre/lox* recombination was not thought possible because immobilized DNA has a different topological structure than either native DNA *in vivo* or purified DNA *in vitro*. The *Cre/lox* reaction changes the topological structure of the DNA substrate. A DNA structure tethered to a solid support might not undergo the conformational changes required for recombination. One of ordinary skill in the art would have thought *Cre/lox* recombination was inhibited or impossible on a solid support.

See Bennett Declaration at APPENDIX B. Thus, there is no reasonable expectation of success where **topology is known to be critical to recombinase function**.

A person of ordinary skill in the art has declared under penalty of law that because recombinases are topologically sensitive he would not predict that they would function on a tethered substrate on a solid support. This **fact** has not been rebutted.

xii) Examiner Summarily Dismisses Declaratory Evidence

Examiner states that the declaration was unpersuasive because it "failed to provide evidence of unexpected results."[6] Examiner also asserts that Applicants "fail to provide any data[7] or evidence to support the Declaration."

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[6] Advisory Action, 10/16/07.

Examiner is incorrect both in fact and as a matter of law.

The Declaration **itself** is competent evidence, **and** it is **supported by copious art** showing the topological sensitivity of the recombinase. The conclusion that one would predict that recombinase would not function on a tethered substrate **follows rationally** from the **fact** that recombinase is topologically sensitive.

This is not a case of providing a merely conclusory legal opinion that is unsupported by facts. *In re Alton*, 76 F.3d 1168, 1174 (Fed. Cir. 1996) ("the examiner's final rejection and Answer contained two **errors**: (1) viewing the Wall Declaration as opinion evidence addressing a question of law rather than a question of fact; and (2) the summary dismissal of the declaration, **without an adequate explanation** of why the declaration failed to rebut the Board's *prima facie* case of inadequate description.").[8]

Rather, the Bennett Declaration offers a factual rationale to explain why one of ordinary skill in the art would have predicted a lack of success based on the known topological requirements of the enzyme. *Id.* at 1174-75 ("the declaration is offering factual evidence in an attempt to explain why one of ordinary skill in the art would have understood the specification to describe [the invention]. . . . the examiner's dismissal of the declaration on the grounds that "little weight is given an opinion affidavit on the ultimate legal question at issue" was **error**.").

xiii) UNCHALLENGED EVIDENCE WINS

Applicants have provided competent evidence showing non-obviousness and Examiner has not countered with any evidence. Examiner has had ample opportunity to present competent evidence, but has refused to do so.

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[7] The specification is actually replete with data showing unexpected results—it was unexpected that the recombinase would function on tethered substrates and the data in the specification shows that it does.

[8] *See* also *MPEP* 716 (noting that in rejecting 132 declarations the Examiner must "include a **detailed explanation** of the reasons why the affidavit or declaration is insufficient."); *see* also *MPEP* 716.01 (Evidence traversing rejections, when timely presented, must be considered by the examiner whenever present. . . . Where the evidence is insufficient to overcome the rejection, the examiner must specifically explain why the evidence is insufficient. **General statements** such as "the declaration lacks technical validity" or "the evidence is not commensurate with the scope of the claims" **without an explanation supporting such findings are insufficient**.") (emphasis added).

This is a simple case of some evidence versus no evidence, and there can only be a single conclusion in favor of unchallenged evidence. *In Re John B. Sullivan, et al.* (Fed. Cir. 2007) ("The claimed composition cannot be held to have been obvious **if competent evidence rebuts the *prima facie* case** of obviousness. By failing to consider the submitted evidence, the Board thus committed **error.**") (emphasis added); *In re Rinehart*, 531 F.2d 1048 (C.C.P.A. 1976) (reversing a finding of obviousness where there was no reasonable expectation that a process combining the prior art steps could be successfully scaled up in view of **unchallenged evidence** showing that the prior art processes individually could not be commercially scaled up successfully) (emphasis added); *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463 (Fed. Cir. 1984) ("On the **unchallenged evidence** of record, we are left with a 'definite and firm conviction' that the district court's finding of anticipation was mistaken and therefore clearly erroneous.") (emphasis added); *Grepke v. General Electric Co.*, 280 F.2d 508, 511 (7th Cir. 1960) ("in the face of the positive denial of such fact by **unchallenged evidence**, the Trial Court should have accepted its responsibility and directed a verdict for the defendant as a **matter of law**.") (emphasis added).

xiv) COMBINED ART TEACHES A DIFFERENT INVENTION

Finally, if one combines the cited art, what is actually obtained is not the invention, but rather:

> a) Use of a modified nucleotide and a repair enzyme to generate a 3' overhang for use in cloning strategies,
>
> b) together with use of the *Cre/lox* system to combine two vectors each having a recombinase site and **one of which must have a conditional origin of replication,** and
>
> c) solid phase assembly where the DNA is **first eluted** from the solid support, and **then circularized**.

Examiner has articulated no rationale for selecting the desired elements, nor any rationale for leaving behind the undesired elements. Examiner's sole rationale is that the modifications will result in "increased efficiency"[9] and will "stabilize and control the assembly"[10] and that the

[9] Office Action, 7/27/06, p. 4.

"references are in the field of Applicant's endeavor and are pertinent to improving gene assembly."[11] However, such rationale does not inform us as to **which** elements to cherry-pick and which to ignore. Indeed, it is only with the teachings of the invention that one knows which elements to retain, and which to dispose of as not needed. *Marconi Wireless Tel. Co. v. United States*, 320 U.S. 1, 62 (1943) ("Reconstruction by hindsight, making obvious something that was not at all obvious to superior minds until someone pointed it out, -- this is too often a tempting exercise for astute minds."); *KSR Int'l Co.,* 127 S. Ct. at 1742 (U.S. 2007) ("A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning.").

[10] Office Action, 7/27/06, p. 5.

[11] Office Action, 7/27/06, p. 6.

8. CONCLUSION

None of the cited art shows simultaneously removing and circularizing assembled PCR fragments from a solid support with a recombinase. A *prima facie* obviousness case cannot be maintained if the art does not teach every element of the invention.

Even if the missing elements were available (and they are not since recombinases have never before been used on a solid support), one must also show a reasonable expectation of success in making the combination or modification. The expectation of success cannot be pulled from thin air, but must be substantiated to make a *prima facie* case of obviousness.

Applicants have provided 1) published facts, 2) a well articulated rationale, and 3) Declaratory facts as to why there was no reasonable expectation of success. All such evidence has been summarily disregarded as "unpersuasive" without any rebuttal facts or explanation. Thus, Applicants evidence remains unchallenged by competent evidence.

Finally, the combined art does not even lead to the invention, but to something entirely different.

Therefore, for at least these reasons, the Examiner has not proven a *prima facie* case of obviousness and/or Applicants have rebutted same.

Fees for the Appeal Brief were previously filed and no additional fees are believed to be due for this response. However, should there be any additional fees required, please charge such additional fees to Deposit Account No. 50-3420 (reference 31175413-002002 TV).

Dated: April 11, 2008

Respectfully submitted,

BAKER & MCKENZIE LLP

By /Tamsen Valoir/

Tamsen Valoir, PhD
Registration No.: 41,417
Pennzoil Place, South Tower
711 Louisiana, Suite 3400
Houston, Texas 77002-2746 USA
Tel: +1 713 427 5007
Fax: +1 713 427 5099
Email: Tamsen.Valoir@bakernet.com
Attorneys For Applicants

APPENDIX A: CLEAN COPY OF CURRENT PENDING CLAIMS

1. (previously presented): A method of assembling PCR fragments, comprising;

a) making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and wherein the first PCR fragment comprises a first site specific recombinase site;

b) treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang and immobilizing the first PCR fragment on a solid support or vice versa;

c) making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang;

d) treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang;

e) annealing and ligating the first and second PCR fragments;

f) optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises a second site specific recombinase site; and

g) simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step.

Claim 2 (original): The method of claim 1, where one of the PCR fragments comprises an origin of replication and a selectable marker.

Claim 3 (original): The method of claim 1, wherein the first PCR fragment or the last PCR fragment comprises an origin of replication and a selectable marker.

Claim 4 (original): The method of claim 1, wherein the site specific recombinase is CRE and the site specific recombinase site is lox.

Claim 5 (original): The method of claim 1, wherein the nucleotide is deoxyuridine and the DNA repair enzyme is uracil-DNA-glycosylase followed by T_4 endonuclease V.

Claim 6 (original): The method of claim 5, wherein the assembled DNA is greater than 30 kb.

Claim 7 (original): The method of claim 5, wherein the assembled DNA is greater than 30, 40, 50, 75, 100, 125, 150, 200, 250 , 300, 350, 400, 450, 500, 750, 1000 or 1500 kb.

APPENDIX B: EVIDENCE

Bennett Declaration: This declaration was included in the Response to Office Action filed May 17, 2007 and entered into evidence by the examiner.

Kilbride E.A., et al., Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system, J Mol Biol. 355(2):185-95 (2006): A selected section of this article was included in the Response to Office Action filed September 26, 2007 and entered into evidence by the examiner.

Vetcher A.A., et al., DNA topology and geometry in Flp and Cre recombination, J Mol Biol. 357(4):1089-104 (2006): A selected section of this article was included in the Response to Office Action filed September 26, 2007 and entered into evidence by the examiner.

Grainge I., et al., Symmetric DNA sites are functionally asymmetric within Flp and Cre site-specific DNA recombination synapses, J Mol Biol. 320(3):515-27 (2002): A selected section of this article was included in the Response to Office Action filed September 26, 2007 and entered into evidence by the examiner.

Crisona N.J., et al., The topological mechanism of phage lambda integrase, J Mol Biol. 18;289(4):747- 75 (1999): A selected section of this article was included in the Response to Office Action filed September 26, 2007 and entered into evidence by the examiner.

Kilbride E., et al., Topological selectivity of a hybrid site-specific recombination system with elements from Tn3 res/resolvase and bacteriophage P1 loxP/Cre. J Mol Biol. 289(5):1219-30 (1999): A selected section of this article was included in the Response to Office Action filed September 26, 2007 and entered into evidence by the examiner.

Advisory Action: This action was mailed October 16, 2007 and entered into evidence by the examiner.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No.	:	10/699,511	Confirmation No. 3571
Applicant	:	Bennett, George N.	
Filed	:	October 31, 2003	
TC/A.U.	:	1637	
Examiner	:	Calamita, H.	
Docket No.	:	31175413-002002	
Customer No.	:	51738	
Entitled	:	Method for assembling PCR fragments of DNA	

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GEORGE N. BENNETT UNDER 37 CFR §1.132

I, George N. Bennett, Declare as follows:

I am at least 18 years of age and am competent in all respects to make the following statements.

I am a joint inventor for claims 1-7 currently pending in US Patent Application No. 10/699,511.

I have read and understand the above-referenced application and pending claims.

I am a person of ordinary skill in the art of assembling nucleic acids including assembling PCR fragments with recombinases, see the attached *curriculum vitae.*

The present invention is the **first** demonstration of removal and circularization of a previously linear DNA from a solid support using a recombinase. *Cre/lox* recombination is affected by three factors: the method of release from the solid support, the length of the DNA to be recombined and relative concentrations of both the DNA and active Cre enzyme. Prior to this invention, it was not known if the Cre enzyme would release the DNA from solid support or if the DNA would be able to undergo further processing. It was not known if length of DNA attached to the solid support would affect Cre activity. Finally, the relative concentration of Cre

enzyme required for activity under these conditions was unknown. Therefore it was not known if the Cre enzyme would work efficiently with a linear DNA substrate attached to a solid support.

Prior to the present invention, the ability of the CRE protein to function on immobilized DNA was unknown. The use of immobilized DNA for the *Cre/lox* recombination was not thought possible because immobilized DNA has a different topological structure than either native DNA *in vivo* or purified DNA *in vitro*. The *Cre/lox* reaction changes the topological structure of the DNA substrate. A DNA structure tethered to a solid support might not undergo the conformational changes required for recombination. One of ordinary skill in the art would have thought *Cre/lox* recombination was inhibited or impossible on a solid support.

I further declare that all statements made herein of my own knowledge are true and made on information believed to be true; further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of any application for which it is used.

Dated: May 8 2007

By _____

George N. Bennett
Dept. of Biochemistry and Cell Biology
Rice University
P.O. Box 1892 MS 140
Houston, TX 77251

GEORGE N. BENNETT

TITLE: Chair and E. Dell Butcher Professor of Biochemistry and Cell Biology

EDUCATION: University of Nebraska, Lincoln, Nebraska, B.S.,1968 (Chemistry)
Purdue University, West Lafayette, Indiana, Ph.D., 1974 (Biochemistry)
Stanford University, Palo Alto, California, Postdoctoral, 1975 - 1978

RESEARCH AND PROFESSIONAL EXPERIENCE:

1. Chair, Biochemistry & Cell Biology, Rice University, 2003-present.
2. Professor of Biochemistry, Rice University, Houston, Texas, 1992-present.
3. Associate Professor of Biochemistry, Rice University, Houston, Texas, 1984-1992.
4. Assistant Professor of Biochemistry, Rice University, Houston, Texas, 1978-1984.
5. Postdoctoral Research Fellow, Department of Biological Sciences, Stanford University, 1975-1978.
6. Graduate Student, Department of Biological Sciences, Purdue University, 1968-1969 and 1971-1974.
7. Laboratory Technician, military service, Medical Biochemistry Laboratory, LAIR, Presidio, San Francisco, California, 1969-1971.
8. Research Assistant, USDA, Northern Regional Research Laboratory, Peoria, Illinois, 1968.

HONORS and RECOGNITION:

University of Nebraska Foundation Scholarship
University of Nebraska Regents Scholarship
Phi Lambda Upsilon-Merck Award
Pi Mu Epsilon
Phi Eta Sigma
European Molecular Biology Organization Conference Fellowship (1973)
National Institutes of Health Postdoctoral Research Fellowship (Stanford University, 1975-1978)
Elected Member of American Society of Biological Chemists (1981)
American Men and Women of Science
Outstanding Faculty Award, Rice Premedical Society, 1995
Outstanding Associate, Lovett College, 1995,
Cain Project Highlighted Teacher for Innovation 2003
Outstanding Associate, Lovett College, 2004
Distinguished Associate (Award for student advising and mentoring) 2002
Distinguished Associate (Award for student advising and mentoring) 2003
Hershel M. Rich Invention Award 2004
Hershel M. Rich Invention Award, 2005
Hamill Innovation Award, 2005
Fellow American Academy of Microbiology (2006)
E. Dell Butcher Professor of Biochemistry & Cell Biology

EDITORAL BOARDS:

Editorial Board, *Anaerobe*, 1994-2005
Editorial Board, *Applied Microbiology and Biotechnology*, 2002-2006
Editorial Board, *Electronic Journal of Biotechnology*, 1998-2006

SERVICE

A. University Service

1. University Activities

Member, Campus Safety Committee, 1984-1989
Member, Campus Safety Officer Recruiting Committee, 1989-1991
Member, Environmental Health and Safety Working Group, 1991-1993
Member, Strategic Planning Committee, Wiess School of Natural Sciences, Undergraduate Studies, 1996-1997
Member, Strategic Planning Committee for the Rice School, 1996-1997
Member, Wiess Scholars Program, 1994-1997
Member, McNair Scholars Program, 1998
Member, BioEngineering Program, 1994-1998
Member, Task Force on Graduate Student Teaching, 1999
Member, Admissions Committee, 1993-1996, 1998-2005
Member, Advisory Committee on Academic Advising, 1999-2000
Member, University Parking Study Advisory Committee, 1999-2000
Co-Chair, Admissions Committee, 2000-2001
Natural Sciences Advisor 1988-2005
Member, Financial Aid Appeal Committee, 2002-2005
Member, Athletic Admission Committee, 2002-2005
Faculty Mentor, Century Scholars Program, 2003-2005
Member, Training Committee NSF IGERT Program, 2001-2005
Member, Natural Sciences Faculty K-12 Resources Committee, 2004-2005
Member, Institute of Biosciences & Bioengineering Steering Committee, 2003-
Member, NIH Biotechnology Training Grant Steering Committee, 2004-

B. Professional Service

Member, Alcohol Fuels Review Panel, U.S. Department of Agriculture, 1989-1991
Panelist, National Science Foundation Biotechnology, 1987, 1992-1993
Panelist, Life in Extreme Environments, NSF Interagency Grant Panel (LExEn), 1998
Panelist, DOE Energy Biosciences
Organizing Committee for International Clostridium 2000 Meeting, Urbana, Illinois, 1999-2000
Organizing Committee for International Clostridium 1994 Meeting, Northwestern University, 1994
Organizer 9th International Clostridium Meeting, Rice University, 2006
Institutional Review Board, The Methodist Hospital Research Institute 2005-
Member, Preceptor Committee, 1 Fellow, M.D. Anderson Cancer Institute, 2000-2002
Member, American Society for Microbiology, 1983-2004

Member, American Chemical Society, 1983-2004
Member, American Society for Biochemistry and Molecular Biology, 1983-2004
Member, American Association for the Advancement of Science, 1992-2004
Member, Society for Industrial Microbiology, 1995-2004 (Symposium session co-organizer, 1994-1996)
Invited Participant, Stanford University Microbial Genome Initiative, 1994-1996
Invited Panel Participant, Biofuels, Panel, USDA, 1994-1996
Reviewer, The Consortium for Plant-Biotechnology Research grants, 1998-1999, 2003-2005
Reviewer, Department of Energy grants, 1992-2000, 2005-6
Reviewer, U.S. Department of Agriculture grants, 1992-2004
Reviewer, Army Research grants, 2001-2006
Reviewer, National Science Foundation grants, 1992-1999, 2001-2006
Reviewer, Applied Microbiology and Biotechnology, Journal of Clinical Microbiology, Applied and Environmental Microbiology, Biotechnology and Applied Biochemistry, Metabolic Engineering, Chemosphere, Biochemistry, Environmental Science & Technology, Journal of Bacteriology, Nature Reviews Microbiology, Biotechnology & Bioengineering, Process Biochemistry, BioMed Central Microbiology, FEMS Microbiology Letters, Biotechnology Progress, Journal of Industrial Microbiology & Biotechnology, Molecular Microbiology, Nucleic Acid Research (in 2004-05 period)
Member, Graduate Ph.D. Thesis Committee, U.T. Medical School, Houston, 1993-1996
Member, Graduate Ph.D. Thesis Committee, St. John's University, 1993-1997
Outside Thesis Reviewer, University of Capetown, South Africa, 1998
External Advisory Committee for PPG, Stanford University -- Martin Brown "Development of New Hypoxic Cytotoxins for Cancer Therapy", 2003-2005
Member, BioHouston Genomics Task Force, 2004-05
Hosting researchers displaced by Allison (2001) and Katrina (2005)
Judge, Houston Science & Engineering Fair (usually team leader), 1991-2004
Judge, Odyssey of the Mind, Houston and State Competitions, 2001-2005
Collaborations with: K.-Y. San -- Department of Bioengineering, Rice University; N. Mantzaris -- Department of Chemical Engineering, Rice University; K. Zygourakis -- Department of Chemical Engineering, Rice University; Praveen V. Vadlani -- AgRenew, Inc; Joseph B. Hughes -- Department of Civil and Environmental Engineering, Georgia Tech; Farrukh Ahmad -- Groundwater Services, Inc.; Carl C. Zhang -- School of Natural and Applied Sciences, University of Houston, Clear Lake; S. Cox -- Department of Computational and Applied Mathematics, Rice University; Martin Brown -- Stanford; Yun Oh -- MD Anderson Cancer Center, Houston; E. T. Papoutsakis – Northwestern

GRADUATE STUDENT SUPERVISION:

Current Graduate Students:

Name:	**Degree Sought:**
Sullivan, Leighann	Ph.D.

Past Graduate Students:

Elizabeth A. Auger, Ph.D., "Studies on the Effects of Temperature, pH, and Anaerobiosis on Gene Expression in *E. coli* K-12," 1988, currently Faculty, Department of Biology, St. Joseph's College, Standish, Maine

Ed Belouski, M.A., "Cloning and Sequencing of Genes Involved in Glycolysis from *Clostridium actobutylicum*," 1996 currently, DNA testing, Texas State Fish & Game Department, Palacios, Texas

Richard B. Gayle, III, Ph.D., "Construction and Characterization of *Escherichia coli* Plasmids Useful in the Manipulation of DNA," 1984, currently Research Scientist, Immunex Corp., Seattle, Washington

George L. Herrin, Jr., Ph.D., "The Effects of Altered Supercoiling on Expression from Bacterial Promoters," 1985, currently Director, State DNA Analysis Laboratory, Atlanta, Georgia

Shi-Yuan Meng, Ph.D., "Studies on the *cad* Operon of *Escherichia coli* K-12: A pH Regulatory System in Bacteria," 1992, currently Research Scientist, Amgen Inc., Thousand Oaks, California

Daniel J. Petersen, Ph.D., "Characterization of the Acetone Production Pathway Genes from *Clostridium acetobutylicum* ATCC 824," 1991, currently CODIS Administrator, Oregon State Police Crime Laboratory, Portland OR

David R. Russell, Ph.D., "Construction and Analysis of *Escherichia coli* Hybrid and Variant Promoters," 1983, currently Director of Plant Molecular Biology, Renessen, Chicago Il,

Miles Scotcher, Ph.D., "Genetic Factors Affecting the Regulation of Solventogenesis in *Clostridium acetobutylicum* ATCC 824," 2004, currently Postdoctoral Fellow, University of Washington, Seattle

Xiao-Lu Shi, Ph.D., "Studies on the Regulation of Biodegradative Arginine and Lysine Decarboxylase Gene Expression in *Escherichia coli*," 1995, currently in Shanghai, China after Rockefeller and UT-Southwestern.

Tara Soughers, M.A., "Study of the Effect of DNA Sccondary Structure on Reactivity of Hedamycin and Analysis of Systems for Directed Mutagenesis," 1985, currently in the Ministry in Moravia, New York

Polly S. Vermersch (Ledvina), Ph.D., "Genetic Strategies for Analyzing Proteins: Applications Utilizing the R388 Type II Dehydrofolate Reductase," 1988, currently Architect in private practice, Houston, Texas

Stephanie Wardwell, Ph.D., "Acetoin in *Clostridium acetobutylicum* ATCC 824," 1999, currently Patent agent, Washington, D.C.

John Wong, Ph.D., "Genetic Regulation in *Clostridium acetobutylicum* ATCC 824," 1995, Postdoctoral Fellow, Oregon Health Science Center, Portland

POSTDOCTORAL SUPERVISION:

Name:	**Year(s) Supervised:**
M. Ali	2001 – 2004
J. Cary	1987-1989
E. Green	1993-1997
G. Herrin	1985
K. Huang	1998 – 1999
S. Huang	1998
R. Kutty	2001–present
B. Lu	1998-1999
M. Lyristis	1997–1999
P. D. Miller	1981-1984

M. Peredelchuk	1995-1998
R. Padda	1998-2000
C. Sass	1990-1991
K. Stim	1989-1995
M. Tyurin	1998-1999
Y-T. Yang	1999–2001
K. Yesland	1996-1997
J. Wong	1995
Y. Zhao	2000- 2003
Sagit Shalel-Levanon	2003 -2005
Y. C. Park	2004-2006
T. B. Causcy	2004-2006

VISITING FACULTY (SABBATICAL) SUPERVISION:

P. Lindahl	**1996**
H.Y. Song	**2002-2003**

PUBLICATIONS:

1. Bennett, G.N., Mackey, J.K., Weibers, J.L., and Gilham, P.T., 2'-*O*-(α-methoxylethyl)-nucleoside 5'-diphosphates as "single-addition" substrates in the synthesis of specific oligoribonucleotides with polynucleotide phosphorylase, *Biochemistry*, **12**, 3956-3962 (1973).
2. Sninsky, J.J., Bennett, G.N., and Gilham, P.T., "Single-addition" and "transnucleotidation" reactions catalyzed by polynucleotide phosphorylase. Effect of enzymatic removal of inorganic phosphate during reaction, *Nucleic Acids Research*, **1**, 1665-1674 (1974).
3. Bennett, G.N., The use of the methoxyethyl blocking group in the enzymatic synthesis of specific oligonucleotides and in the chemical synthesis of guanosine tetraphosphate, Ph.D. Thesis, Purdue University (1974).
4. Bennett, G.N., and Gilham, P.T., "Single-addition" substrates for the synthesis of specific oligoribonucleotides with polynucleotide phosphorylase. Synthesis of 2'-*O*-(α-methoxylethyl)-nucleoside 5'-diphosphates, *Biochemistry*, **14**, 3152-3158 (1975).
5. Sninsky, J.J., Hawley, D.M., and Bennett, G.N., Modifications in the sugar moieties of nucleoside diphosphates that result in limited additions to oligonucleotide primers in polynucleotide phosphorylase reactions, *Fed. Proc.*, **34**, 702 (Abstract No. 2742) (1975).
6. Yanofsky, C., Korn, L., Lee, F., Bertrand, K., Bennett, G., and Schweingruber, M., The two transcription control sites in the tryptophan operon of *E. coli*, *Fed. Proc.*, **35**, 1343 (1976).
7. Bennett, G.N., Schweingruber, M.E., Brown, K.D., Squires, C., and Yanofsky, C., Nucleotide sequence of region preceding *trp* mRNA initiation site and its role in promoter and operator function, *Proceedings of the National Academy of Sciences U.S.A.*, **73**, 2351-2355 (1976).
8. Bennett, G.N., Gough, G.R., and Gilham, P.T., Guanosine tetraphosphate and its analogs. Chemical synthesis of guanosine 3',5'-di(pyrophosphate), deoxyguanosine 3',5'-di(pyrophosphate), guanosine 2',5'-bis(methylenediphosphonate), and guanosine 3',5'-bis(methylenediphosphonate), *Biochemistry*, **15**, 4623-4628 (1976).
9. Bennett, G.N., Brown, K.D., and Yanofsky, C., Nucleotide sequence of the promoter-operator region of the tryptophan operon from *E. coli* and *S. typhimurium*, *Fed. Proc.*, **36**, 878 (1977).
10. Franklin, N., and Bennett, G.N., DNA sequencing of the N gene of bacteriophage lambda, *Abstracts, Cold Spring Harbor Meeting on Bacteriophage and Single-Stranded DNA Phage*, **39** (1977).

11. Hawley, D.M., Sninsky, J.J., Bennett, G.N., and Gilham, P.T., Activity of polynucleotide phosphorylase with nucleoside diphosphates containing sugar ring modifications, *Biochemistry*, **17**, 2082-2086 (1978).
12. Bennett, G.N., Schweingruber, M.E., Brown, K.D., Squires, C., and Yanofsky, D., Nucleotide sequences of the promoter-operator region of the tryptophan operon of *Escherichia coli*, *J. Mol. Biol.* **121**, 113-137 (1978).
13. Bennett, G.N., Brown, K.D., and Yanofsky, C., Nucleotide sequence of the promoter-operator region of the tryptophan operon of *Salmonella typhimurium*, *J. Mol. Biol.*, **121**, 139-152 (1978).
14. Brown, K.D., Bennett, G.N., Lee, F., Schweingruber, M.E., and Yanofsky, C., RNA polymerase interaction at the promoter-operator region of the tryptophan operon of *Escherichia coli* and *Salmonella typhimurium*, *J. Mol. Biol.*, **121**, 153-177 (1978).
15. Bennett, G.N., and Yanofsky, C., Sequence analysis of operator constitutive mutants of the tryptophan operon of *Escherichia coli*, *J. Mol. Biol.*, **121**, 179-192 (1978).
16. Lee, F., Bertrand, K., Bennett, G., and Yanofsky, C., Comparison of the nucleotide sequences of the initial transcribed regions of the tryptophan operons of *Escherichia coli* and *Salmonella typhimurium*, *J. Mol. Biol.*, **121**, 193-217 (1978).
17. Stauffer, G.V., Zurawski, G., and Bennett, G.N., *In vivo* cloning of DNA regions carrying mutations linked to selectable genes: Application to mutations in the regulatory region of the *Escherichia coli* tryptophan operon, *Plasmid*, **2**, 498-502 (1979).
18. Franklin, N.C., and Bennett, G.N., The N-protein of bacteriophage lambda, defined by its DNA sequence, is highly basic, *Gene*, **8**, 107-119 (1979).
19. Manly, S.P., and Bennett, G.N., Effects of the tryptic core of *lac* repressor on the methylation pattern of the operator DNA sequence, *Fed. Proc.*, **39**, 1607 (1980).
20. Nichols, B.P., Miozzari, G.F., van Cleemput, M., Bennett, G.N., and Yanofsky, C., Nucleotide sequences of the *trp* G regions of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium* and *Serratia marcescens*, *J. Mol. Biol.*, **142**, 503-517 (1980).
21. Oppenheim, D.S., Bennett, G.N., and Yanofsky, C., *Escherichia coli* RNA polymerase and *trp* repressor interaction with the promoter-operator region of the tryptophan operon of *Salmonella typhimurium*, *J. Mol. Biol.*, **144**, 133-142 (1980).
22. Sumner, W., II, and Bennett, G.N., Anthramycin inhibition of restriction endonuclease cleavage and its use as a reversible blocking agent in DNA constructions, *Nucleic Acids Research*, **9**, 2105-2119 (1981).
23. Russell, D.R., and Bennett, G.N., Characterization of the β-lactamase promoter of pBR322, *Nucleic Acids Research*, **9**, 2517-2533 (1981).
24. Russell, D.R., and Bennett, G.N., Cloning of small DNA fragments containing the *Escherichia coli* tryptophan operon promoter and operator, *Gene*, **17**, 9-18 (1982).
25. Russell, D.R., and Bennett, G.N., Analysis of *in vitro* constructed *E. coli* promoters, *Fed. Proc.*, **41**, 758 (1982).
26. Herrin, G.L., Jr., Russell, D.R., and Bennett, G.N., A stable derivative of pBR322 conferring increased tetracycline resistance and increased sensitivity to fusaric acid, *Plasmid*, **7**, 290-293 (1982).
27. Bennett, G.N., Formation of alkali labile linkages in DNA by hedamycin and use of hedamycin as a probe of protein-DNA complexes, *Nucleic Acids Research*, **10**, 4581-4594 (1982).
28. Russell, D.R., and Bennett, G.N., Construction and analysis of *in vivo* activity of *E. coli* promoter hybrids and promoter mutants that alter the -35 to -10 spacing, *Gene*, **20**, 231-243 (1982).
29. Herrin, G.L., Jr., and Bennett, G.N., The effect of supercoiling on expression from a series of bacterial fusion promoters, *Fed. Proc.*, **42**, 2262 (1983).
30. Manly, S.P., Bennett, G.N., and Matthews, K.S., Perturbation of *lac* operator DNA modification by tryptic core protein from lactose repressor, *Proceedings of the National Academy of Sciences U.S.A.*, **80**, 6219-6223 (1983).

31. Russell, D.R., Auger, E.A., Vermersch, P.S., and Bennett, G.N., Role of DNA regions flanking the tryptophan promoter of *Escherichia coli*. I. Insertion of synthetic oligonucleotides, *Gene*, **32**, 337-348 (1984).
32. Herrin, G.L., Jr., and Bennett, G.N., Role of DNA regions flanking the tryptophan promoter of *Escherichia coli*. II. Insertion of *lac* operator fragments, *Gene*, **32**, 349-356 (1984).
33. Manly, S.P., Bennett, G.N., and Matthews, K.S., Enzymatic digestion of operator DNA in the presence of the *lac* repressor tryptic core, *J. Mol. Biol.*, **179**, 335-350 (1984).
34. Russell, D.R., Miller, P.D., and Bennett, G.N., *In vitro* characterization of hybrid promoters and altered tryptophan operon promoters, *Biochemistry*, **24**, 1410-1417 (1985).
35. Gayle, R.B., III, Vermersch, P.S., and Bennett, G.N., Construction and characterization of pBR322-derived plasmids with deletions of the RNA I region, *Gene*, **41**, 281-288 (1986).
36. Vermersch, P.S., Klass, M.R., and Bennett, G.N., Use of bacterial DHFR-II fusion proteins to elicit specific antibodies, *Gene*, **41**, 289-297 (1986).
37. Herrin, G.L., Jr., and Bennett, G.N., The effects of nalidixic acid on expression from related *E. coli* promoters, *Biochem. Biophys. Res. Commun.*, **135**, 411-418 (1986).
38. Auger, E.A., and Bennett, G.N., Temperature optimization of *in vivo* expression from the *E. coli trp* and *trp*::*lac* promoters, *Biotechnology Letters*, **9** 157-162 (1987).
39. Gayle, R.B., III, Auger, E.A., Gough, G.R., Gilham, P.T., and Bennett, G.N., Formation of *Mbo*II vectors and cassettes using asymmetric *Mbo*II linkers, *Gene*, **54**, 221-228 (1987).
40. Vermersch, P.S., and Bennett, G.N., The use of a selectable *Fok*I cassette in DNA replacement mutagenesis of the R388 dihydrofolate reductase gene, *Gene*, **54**, 229-238 (1987).
41. Vermersch, P.S., and Bennett, G.N., Synthesis and expression of a gene for a mini Type II dihydrofolate reductase, *DNA*, **7**, 243-251 (1988).
42. Cary, J.W., Petersen, D.J., Papoutsakis, E.T., and Bennett, G.N., Cloning and expression of *Clostridium acetobutylicum* phosphotransbutyrylase and butyrate kinase genes in *Escherichia coli*, *J. Bacteriol.*, **170**, 4613-4618 (1988).
43. Auger, E.A., and Bennett, G.N., Regulation of lysine decarboxylase activity in *Escherichia coli* K-12, *Arch. Microbiol.*, **15**, 466-468 (1989).
44. Auger, E.A., Redding, K.E., Plumb, T., Childs, L.C., Meng, S.-Y., and Bennett, G.N., Construction of *lac* fusions to the inducible arginine- and lysine decarboxylase genes of *Escherichia coli* K12, *Molecular Microbiol.*, **3**, 609-620 (1989).
45. Clark, S.W., Bennett, G.N., and Rudolph, F.B., Isolation and characterization of mutants of *Clostridium acetobutylicum* ATCC 824 deficient in acetoacetyl-Coenzyme A:acetate/butyrate: Coenzyme A transferase (EC 2.8.3.9) and in other solvent pathway enzymes, *App. Environ. Microb.*, **55**, 970-976 (1989).
46. Cary, J.W., Petersen, D.J., Bennett, G.N., and Papoutsakis, E.T., Methods for cloning key primary metabolic enzymes and ancillary proteins associated with the acetone-butanol fermentation of *Clostridium acetobutylicum*, *Ann. N.Y. Acad. Sci.*, **589**, 67-81 (1990).
47. Brito, R.M.M., Reddick, R., Bennett, G.N., Rudolph, F.B., and Rosevear, P.R., Characterization and stereochemistry of cofactor oxidation by a type II dihydrofolate reductase, *Biochemistry*, **29**, 9825-9831 (1990).
48. Cary, J.W., Petersen, D.J., Papoutsakis, E.T., and Bennett, G.N., Cloning and expression of *Clostridium acetobutylicum* ATCC 824 acetoacetyl-Coenzyme A:acetate/butyrate:Coenzyme A-transferase in *Escherichia coli*, *App. Environ. Microb.*, **56**, 1576-1583 (1990).
49. Petersen, D.J., and Bennett, G.N., Purification of acetoacetate decarboxylase from *Clostridium acetobutylicum* ATCC 824 and cloning of the acetoacetate decarboxylase gene in *Escherichia coli*, *App. Environ. Microb.*, **56**, 3491-3498 (1990).
50. Wang-Bennett, L.T., Liebl, D.J., and Bennett, G.N., Targeted neuronal lesion induced by photosensitizing dyes, *Brain Research*, **534**, 122-128 (1990).

51. Petersen, D.J., Welch, R.W., Rudolph, F.B., and Bennett, G.N., Molecular cloning of an alcohol (butanol) dehydrogenase gene cluster from *Clostridium acetobutylicum* ATCC 824, *J. Bacteriol.*, **173**, 1831-1834 (1991).
52. Vermersch, P.S., and Bennett, G.N., Stability of mutant Type II dihydrofolate reductase proteins in suppressor strains, *J. Biotechnology*, **19**, 49-66 (1991).
53. Hassani, M., Saluta, M.V., Bennett, G.N., and Hirshfield, I.N., Partial characterization of a *lysU* mutant of *Escherichia coli* K-12, *J. Bacteriol.*, **173**, 1965-1970 (1991).
54. Petersen, D.J., and Bennett, G.N., Cloning of the *Clostridium acetobutylicum* ATCC 824 acetyl coenzyme A acetyltransferase (Thiolase; EC 2.3.1.9), *Appl. Environ. Microbiol.*, **57**, 2735-2741 (1991).
55. Petersen, D.J., and Bennett, G.N., Enzymatic characterization of a nonmotile, nonsolventogenic *Clostridium acetobutylicum* ATCC 824 mutant, *Current Microbiol.*, **23**, 253-258 (1991).
56. Petersen, D.J., Welch, R.W., Walter, K.A., Mermelstein, L.D., Papoutsakis, E.T., Rudolph, F.B., and Bennett, G.N., Cloning of an NADH-dependent butanol dehydrogenase gene from *Clostridium acetobutylicum*, *Ann. N.Y. Acad. Sci.*, **646**, 94-98 (1991).
57. Meng, S.-Y., and Bennett, G.N., Nucleotide sequence of the *Escherichia coli cad* operon: A system for detoxification of low extracellular pH, *J. Bacteriol.*, **174**, 2659-2669 (1992).
58. Meng, S.-Y., and Bennett, G.N., Regulation of the *Escherichia coli cad* operon: Location of a site required for acid induction, *J. Bacteriol.*, **174**, 2670-2678 (1992).
59. Mermelstein, L.D., Welker, N.E., Bennett, G.N., and Papoutsakis, E.T., Expression of cloned homologous fermentative genes in *Clostridium acetobutylicum* ATCC 824, *Bio/Technology*, **10**, 190-195 (1992).
60. Lee, S.Y., Bennett, G.N., and Papoutsakis, E.T., Construction of *Escherichia coli-Clostridium acetobutylicum* shuttle vectors and transformation of *Clostridium acetobutylicum* strains, *Biotechnology Letters*, **14**, 427-432 (1992).
61. Tolentino, G.J., Meng, S.Y., Bennett, G.N., and San, K.-Y., A pH-regulated promoter for the expression of recombinant proteins in *Escherichia coli*, *Biotechnology Letters*, **14**, 157-162 (1992).
62. Welch, R.W., Clark, S.W., Bennett, G.N., and Rudolph, F.B., Effects of rifampicin and chloramphenical on product and enzyme levels of the acid- and solvent-producing pathways of *Clostridium acetobutylicum* (ATCC 824), *Enzyme Microbiol. Technol.*, **14**, 277-283 (1992).
63. Hassani, M., Pincus, D.H., Bennett, G.N., and Hirshfield, I.N., Temperature-dependent induction on an acid-inducible stimulon of *Escherichia coli* in broth, *App. Environ. Microbiol.*, **58**, 2704-2707 (1992).
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1. San, K.-Y., Berrios-Rivera, S.J., and Bennett, G.N., Recycling system for manipulation of intracellular NADH availability (patent applied for) (2003).

2. Bennett, G.N., Recombination assembly of large DNA fragments (filed) (2003).

3. Bennett, G.N., and Harrison, M.L., Method for assembly PCR fragments (filed) (2003).

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5. A New Technique to Increase the Intracellular Levels of CoA and Acetyl-CoA in *Escherichia coli* with the Goal of Enhancing Production of Compounds that Require Acetyl-CoA in their Biosynthesis (filed 2003) with R.V. Vadali and K.-Y. San.

6. A New Technique to to Manipulate *Escherichia coli* Metabolic Pathways to Increase Flux through the Intracellular Acetyl-CoA Node and Divert this Flux to Enhance Productivies of Compounds that Require Acetyl-CoA in their Biosynthesis (filed 2003) with R.V. Vadali and K.-Y. San.

7. A Novel Approach to Construct High Molar Succinate Yield Production Strains by Increasing the Intracellular NADH Availability in *Escherichia coli* (filed 2003) with A. Sanchez and K.-Y. San.

8. Aerobic Production of Succinate (filed 2003) with H. Lin and K.-Y. San.

9. Increased Bacterial Acetyl-CoA Pool (filed March 24, 2003) with K.-Y. San and R.V. Vadali.

10. Increasing Intracellular NADPH Availability in *E. coli* (filed 2003) with A. Sanchez and K.-Y. San.

11. Simultaneous Anaerobic Production Of Isoamyl Acetate And Succinic Acid (12/22/04, 12/22/05) 31175413-015001, Ka-Yiu San, Cheryl Dittrich, Ailen Sanchez and George N. Bennett, patent pending

12. High Succinate Producing Bacteria (9/17/04, 9/17/05) 31175413-014001, Ka-Yiu San Henry Lin, Ailen Sanchez and George N. Bennett, patent pending

13. Mutant *E. coli* Strain with Increased Succinic Acid Production (8/17/04, 8/17/05) 31175413-011001, Ka-Yiu San, Ailen Sanchez and George N. Bennett

14. Aerobic Succinate Production in Bacteria (8/9/04, 8/9/05) 31175413-012001, Ka-Yiu San, Henry Lin and George N. Bennett, patent pending

15. "An approach to increase intracellular NADPH availability in Escherichia coli to increase the yield and productivity of NADPH-dependent products" Ka-Yiu San, Henry Lin, Irene Martinez, Jiangfeng Zhu and George N. Bennett

16. "Novel PEPC to Produce Succinate" George N. Bennett, Ka-Yiu San; Mary Lou Harrison

17. "UBICA IN E. Coli" Ka-Yiu San and George N. Bennett

1: J Mol Biol. 2006 Jan 13;355(2):185-95. Epub 2005 Nov 9.

Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system.

Kilbride EA, Burke ME, Boocock MR, Stark WM.

Institute of Biomedical & Life Sciences, University of Glasgow, 56 Dumbarton Road, Glasgow G11 6NU, Scotland, UK.

Many natural DNA site-specific recombination systems achieve directionality and/or selectivity by making recombinants with a specific DNA topology. This property requires that the DNA architecture of the synapse and the mechanism of strand exchange are both under strict control. Previously we reported that Tn3 resolvase-mediated synapsis of the accessory binding sites from the Tn3 recombination site res can impose topological selectivity on Cre/loxP recombination. Here, we show that the topology of these reactions is profoundly affected by subtle changes in the hybrid recombination site les. Reversing the orientation of loxP relative to the res accessory sequence, or adding 4 bp to the DNA between loxP and the accessory sequence, can switch between two-noded and four-noded catenane products. By analysing Holliday junction intermediates, we show that the innate bias in the order of strand exchanges at loxP is maintained despite the changes in topology. We conclude that a specific synaptic structure formed by resolvase and the res accessory sequences permits Cre to align the adjoining loxP sites in several distinct ways, and that resolvase-mediated intertwining of the accessory sequences may be less than has been assumed previously.

PMID: 16303133 [PubMed - indexed for MEDLINE]

1: J Mol Biol. 2006 Apr 7;357(4):1089-104. Epub 2006 Jan 30.

DNA topology and geometry in Flp and Cre recombination.

Vetcher AA, Lushnikov AY, Navarra-Madsen J, Scharein RG, Lyubchenko YL, Darcy IK, Levene SD.

Institute of Biomedical Sciences and Technology and Department of Molecular and Cell Biology, The University of Texas at Dallas, Richardson, TX 75083, USA.

The Flp recombinase of yeast and the Cre recombinase of bacteriophage P1 both belong to the lambda-integrase (Int) family of site-specific recombinases. These recombination systems recognize recombination-target sequences that consist of two 13bp inverted repeats flanking a 6 or 8bp spacer sequence. Recombination reactions involve particular geometric and topological relationships between DNA target sites at synapsis, which we investigate using nicked-circular DNA molecules. Examination of the tertiary structure of synaptic complexes formed on nicked plasmid DNAs by atomic-force microscopy, in conjunction with detailed topological analysis using the mathematics of tangles, shows that only a limited number of recombination-site topologies are consistent with the global structures of plasmids bearing directly and inversely repeated sites. The tangle solutions imply that there is significant distortion of the Holliday-junction intermediate relative to the planar structure of the four-way DNA junction present in the Flp and Cre co-crystal structures. Based on simulations of nucleoprotein structures that connect the two-dimensional tangle solutions with three-dimensional models of the complexes, we propose a recombination mechanism in which the synaptic intermediate is characterized by a non-planar, possibly near-tetrahedral, Holliday-junction intermediate. Only modest conformational changes within this structure are needed to form the symmetric, planar DNA junction, which may be characteristic of shorter-lived intermediates along the recombination pathway.

PMID: 16483600 [PubMed - indexed for MEDLINE]

1: J Mol Biol. 2002 Jul 12;320(3):515-27.

Symmetric DNA sites are functionally asymmetric within Flp and Cre site-specific DNA recombination synapses.

Grainge I, Pathania S, Vologodskii A, Harshey RM, Jayaram M.

Section of Molecular Genetics and Microbiology and the Institute of Cell and Molecular Biology, University of Texas at Austin, 78712, USA.

Flp and Cre-mediated recombination on symmetrized FRT and loxP sites, respectively, in circular plasmid substrates yield both DNA inversion and deletion. However, upon sequestering three negative supercoils outside the recombination complex using the resII-resIII synapse formed by Tn3 resolvase and the LER synapse formed by phage Mu transposase in the case of Flp and Cre, respectively, the reactions are channeled towards inversion at the expense of deletion. The inversion product is a trefoil, its unique topology being conferred by the external resolvase or LER synapse. Thus, Flp and Cre assign their symmetrized substrates a strictly antiparallel orientation with respect to strand cleavage and exchange. These conclusions are supported by the product profiles from tethered parallel and antiparallel native FRT sites in dilution and competition assays. Furthermore, the observed recombination bias favoring deletion over inversion in a nicked circular substrate containing two symmetrized FRT sites is consistent with the predictions from Monte Carlo simulations based on antiparallel synapsis of the DNA partners. (c) 2002 Elsevier Science Ltd.

PMID: 12096907 [PubMed - indexed for MEDLINE]

1: J Mol Biol. 1999 Jun 18;289(4):747-75.

The topological mechanism of phage lambda integrase.

Crisona NJ, Weinberg RL, Peter BJ, Sumners DW, Cozzarelli NR.

Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720, USA.

Bacteriophage lambda integrase (Int) is a versatile site-specific recombinase. In concert with other proteins, it mediates phage integration into and excision out of the bacterial chromosome. Int recombines intramolecular sites in inverse or direct orientation or sites on separate DNA molecules. This wide spectrum of Int-mediated reactions has, however, hindered our understanding of the topology of Int recombination. By systematically analyzing the topology of Int reaction products and using a mathematical method called tangles, we deduce a unified model for Int recombination. We find that, even in the absence of (-) supercoiling, all Int reactions are chiral, producing one of two possible enantiomers of each product. We propose that this chirality reflects a right-handed DNA crossing within or between recombination sites in the synaptic complex that favors formation of right-handed Holliday junction intermediates. We demonstrate that the change in linking number associated with excisive inversion with relaxed DNA is equally +2 and -2, reflecting two different substrates with different topology but the same chirality. Additionally, we deduce that integrative Int recombination differs from excisive recombination only by additional plectonemic (-) DNA crossings in the synaptic complex: two with supercoiled substrates and one with relaxed substrates. The generality of our results is indicated by our finding that two other members of the integrase superfamily of recombinases, Flp of yeast and Cre of phage P1, show the same intrinsic chirality as lambda Int. Copyright 1999 Academic Press.

PMID: 10369759 [PubMed - indexed for MEDLINE]

1: J Mol Biol. 1999 Jun 25;289(5):1219-30.

Topological selectivity of a hybrid site-specific recombination system with elements from Tn3 res/resolvase and bacteriophage P1 loxP/Cre.

Kilbride E, **Boocock MR**, **Stark WM**.

Institute of Biomedical and Life Sciences, University of Glasgow, 56 Dumbarton Road, Glasgow, G11 6NU, Scotland.

In order to investigate the functions of the parts of the Tn 3 recombination site res, we created hybrid recombination sites by placing the loxP site for Cre recombinase adjacent to the "accessory" resolvase-binding sites II and III of res. The efficiency and product topology of in vitro recombination by Cre between two of these hybrid sites were affected by the addition of Tn 3 resolvase. The effects of resolvase addition were dependent on the relative orientation and spacing of the elements of the hybrid sites. Substrates with sites II and III of res close to loxP gave specific catenated or knotted products (four-noded catenane, three-noded knot) when resolvase and Cre were added together. The product topological complexity increased when the length of the spacer DNA segment between loxP and res site II was increased. Similar resolvase-induced effects on Cre recombination product topology were observed in reactions of substrates with loxP sites adjacent to full res sites. The results demonstrate that the res accessory sites are sufficient to impose topological selectivity on recombination, and imply that intertwining of two sets of accessory sites defines the simple catenane product topology in normal resolvase-mediated recombination. They are also consistent with current models for the mechanism of catalysis by Cre.

PMID: 10373363 [PubMed - indexed for MEDLINE]

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/699,511	10/31/2003	George Nelson Bennett	61683-00002USPT	3571

51738 7590 10/16/2007

BAKER & MCKENZIE LLP
Pennzoil Place, South Tower
711 Louisiana, Suite 3400
HOUSTON, TX 77002-2716

EXAMINER	
CALAMITA, HEATHER	
ART UNIT	PAPER NUMBER
1637	

MAIL DATE	DELIVERY MODE
10/16/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Advisory Action Before the Filing of an Appeal Brief	Application No.	Applicant(s)	
	10/699,511	BENNETT ET AL.	
	Examiner	Art Unit	
	Heather G. Calamita, Ph.D.	1637	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 26 September 2007 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:

   a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.

   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☒ The Notice of Appeal was filed on *26 September 2007*. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).

5. ☐ Applicant's reply has overcome the following rejection(s): _____.

6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

7. ☒ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: *1-7*.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).

9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).

10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.

12. ☐ Note the attached Information Disclosure Statement(s). (PTO/SB/08) Paper No(s). _____

13. ☐ Other: _____.

GARY BENZION, PHD
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Heather Calamita
Art Unit 1637

Continuation of 11. does NOT place the application in condition for allowance because: Applicants argue none of the cited art shows "simultaneously removing and circularizing" assembled PCR fragments from a solid support with a recombinase. This argument is not persuasive because there is strong suggestion in the prior art that the combination of these technologies, specifically assembly of DNA fragments on a solid support and simultaneous removal recombination and circularization of the DNA would be successful. Additionally, the federal circuit held in Pharmastem Therapeutics, Inc. v. Viacell, Inc., __ F.3d __ (Fed. Cir. 2007) a treatment method to be obvious citing the following:

1) KSR followed - Confirmation of Stem Cell Properties Obvious: The invention was novel in the sense that it was not confirmed in the prior art that umbilical cord blood is capable of hematopoietic reconstitution. Relying upon KSR, the court majority stated that "[w]hile the inventors may have proved conclusively what was strongly suspected before - that umbilical cord blood is capable of hematopoietic reconstitution - and while their work may have significantly advanced the state of the science of hematopoietic transplantations by eliminating any doubt as to the presence of stem cells in cord blood, the mouse experiments and the conclusions drawn from them were not inventive in nature. Instead, the inventors merely used routine research methods to prove what was already believed to be the case. Scientific confirmation of what was already believed to be true may be a valuable contribution, but it does not give rise to a patentable invention."Applicant argues the previously submitted declaration provides evidence as to unexpected results. This declaration was not persuasive because the declaration failed to provide persuasive evidence as to unexpected results. It is well established in the art that Cre/Lox recombinase will simultaneously recombine and circularize plasmid DNA. It is therefore not unreasonable to expect success when using Cre/lox to simultaneously recombine and circularize DNA which is attached to a substrate. Applicants argue the topology of DNA is affected by binding to a solid support which affects recombinase activity and that topology is known to be critical to recombinase function. Applicant submitts several papers in support of this assertion. None of these papers support the assertion that by binding DNA to a solid substrate the structure is changed so markedly that recombinase would not function.

Applicant argues a recombinase is not a ligase and the assembly of DNA on a solid support using a ligase is not analoguous and cannot be used to extrapolate success for the applicaton of recombinase. This argument is not persuasive because the issue is reasonable expectation of success. There is a reasonable expectation of success because the prior art directly points to the assembly of PCR products (taught by Watson), that assembly can occur on a solid support (taught by Stahl) and that Cre recombinase provides simultaneous recombination and circularization of plasmid DNA in vitro (taught by Elledge). Applicant asserts that one of skill in the art would have thought Cre/lox recombination was inhibited or impossible on a solid support. Applicant fails to provide evidence to this effect. The prior art of record does not indicate this was thought to be the case as Cre/lox is a well known well used system for in vitro recombination. Additionally with regard to Applicant's request for an affidavit, Examiner notes that there is no basis in MPEP § 2144 which requires the Office to supply an affidavit when the motivation is provided directly in the prior art document(s). None of the facts relied upon in the 103 rejection are the personal knowledge of the examiner. Instead, as noted in the 103 rejection recited above, the motivation is expressly stated within the reference(s). The Examiner at no point in the rejection indicated the use of personal knowledge in finding a motivation to combine between the references. This line of argument is inappropriate. Applicant argues the Examiner must consider the declaratory evidence. This argument is not persuasive as the declaration was considered in the Office Action mailed July 26, 2007. It was noted in the Action that Applicant's declaration failed to provide evidence of unexpected results. Applicants merely assert in the declaration that the function of recombinase is unexpected and fail to provide any data or evidence to support the assertion.

APPENDIX C: RELATED PROCEEDINGS

Not applicable